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Salinity effects on survival, growth and morphometry of four Egyptian *Artemia* populations (International Study on *Artemia*. LXVII)

Nagy El-Bermawi[1], Athanasios D. Baxevanis[2,*], Theodore J. Abatzopoulos[2], Gilbert Van Stappen[1] & Patrick Sorgeloos[1]

[1]*Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Rozier 44, B-9000 Gent, Belgium*
[2]*Department of Genetics, Development and Molecular Biology, School of Biology, Faculty of Sciences, Aristotle University of Thessaloniki, 541 24 Thessaloniki, Greece*
*(*Author for correspondence: Tel.: +30-2-31-099-8301, Fax: +30-2-31-099-8256, E-mail: tbaxevan@bio.auth.gr)*

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Abstract

Four *Artemia* populations from northern Egypt, a bisexual one from Wadi El-Natrun Lake, two coastal parthenogenetic ones from Borg El-Arab and El-Max saltworks and an inland parthenogenetic form from Qarun Lake, were assayed for their survival, growth and morphometric responses measured in laboratory experiments at salinities of 35, 80, 120, 150 and 200 g l^{-1}. The survival rate was determined using regression analysis and analysis of covariance. The bisexual population (Wadi El-Natrun: WN) exhibited its best survival at 80 g l^{-1}. All parthenogenetic strains studied performed similarly (in terms of survival) at all salinities investigated. The population growth rates were based on Von Bertalannfy's equation. In all salinities, WN population had the lowest growth rate (based on K values) among all *Artemia* populations tested. It is obvious that parthenogenetic populations tolerate a broader range of salinities compared to the bisexual one. The two coastal asexual strains had similar survival, growth and morphometric characters at all salinities. Discriminant function analysis based on specific morphometric parameters permitted the assignment of adult *Artemia* individuals to their population of origin with a score as high as 84.6%. Furthermore, specific morphometric parameters (such as the furcal length and the number of setae in each furcal branch) are suitable discriminating characters among the populations studied.

Introduction

The genus *Artemia* (Crustacea: Anostraca) is a complex of bisexual and parthenogenetic species, which are morphologically very similar. The bisexual species are grouped in (1) the New World species, i.e., *Artemia franciscana* (North, Central and South Americas and worldwide as the most invasive species) and *A. persimilis* (mainly in South America), and (2) the Old World species, i.e., *A. salina* that inhabits mainly the Mediterranean basin and adjacent regions, *A. urmiana* (found only in Urmia Lake, Iran), *A. sinica* (China and neighboring countries), *Artemia* sp. from Kazakhstan and *A. tibetiana* (restricted on Tibet's high Plateau) (Abatzopoulos et al., 2002b). The parthenogenetic forms (found only in the Old World) are grouped controversially under the binomen *A. parthenogenetica* but the use of this name is best avoided (see Abatzopoulos et al., 2002a).

The brine shrimp *Artemia* has a diverse geographical distribution and inhabits both inland and coastal saline and hypersaline lakes (Triantaphyllidis

et al., 1998; Van Stappen, 2002). It is found in environments varying considerably in terms of (1) climatic conditions, ranging from humid-subhumid to arid (Vanhaecke et al., 1987), (2) altitude and (3) water anionic composition (Triantaphyllidis et al., 1998). *Artemia* may inhabit chloride, sulphate or carbonate waters and/or combinations of two or even three anions (Bowen et al., 1985, 1988; Lenz, 1987).

Artemia is of considerable economic importance in fish and shellfish larviculture (Bengtson et al., 1991; Dhont & Sorgeloos, 2002). In the recent years, there has been a worldwide effort to discover new *Artemia* strains and characterize them with regard to their potential for applications in aquaculture.

As part of this effort, *Artemia* cysts were collected from two coastal saltworks (Borg El-Arab and El-Max), an inland saltworks (Qarun) and one inland saline lake (i.e., Wadi El-Natrun), all situated in northern Egypt (Fig. 1).

The coastal Borg El-Arab (31° 05′ N, 30° 03′ E) and El-Max (31° 08′ N, 30° 07′ E) saltworks are located along the Mediterranean coast, west of the city of Alexandria (Borg El-Arab 55 km south west and El-Max 15 km west) and cover surface areas of 29 and 39 km^2, respectively. The Borg El-Arab saltworks was constructed in 1981 whereas the El-Max saltworks has been operated since 1759. Qarun Lake (29° 30′ N, 30° 30′ E, Faiyum Province) is a closed depression located at about 85 km southwest of Cairo. The lake is an extended

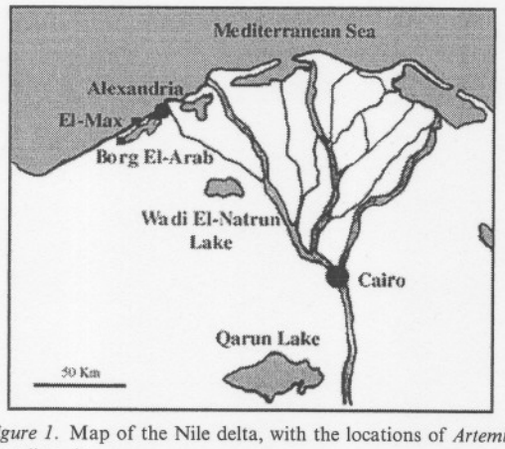

Figure 1. Map of the Nile delta, with the locations of *Artemia* sampling sites.

complex of saline water bodies covering a surface of 214 km^2 with a maximum depth of 8.5 m (Dowidar, 1981; Abdel-Malek et al., 1990). It used to be a fresh water reservoir fed by the Nile (surrounded by agricultural lands) with many fresh water species; due to the high rate of evaporation the salinity of the lake has progressively increased reaching a maximum of 38 g l^{-1} (Bishai & Kirollus, 1980; Dowidar, 1981; Abdel-Malek et al., 1990).

Marine fish species were introduced in 1928 and several transplantations took place in years 1938, 1943, 1945, 1948 and 1970. In 1977, penaeid shrimp larvae and post larvae were successfully introduced and acclimatized (Abdel-Malek et al., 1990). The west side of the lake, where *Artemia* is found, is managed for sodium chloride and sodium sulphate production (Estefan et al., 1980).

Wadi El-Natrun (30° 10′ N, 30° 27′ E) is one of the depressions of the Western Desert of Egypt. It is a complex of saline lakes that stretch over 60 km; the closest is 40 km northwest of Cairo. The bed of this depression is 23 m below sea level. The water source is attributed to the inflow of groundwater. The area experiences extremely arid climatic conditions, i.e., low rainfall, long dry summers and therefore, high rates of evaporation (see El-Sherif, 1989). Several of Wadi Natrun lakes have alkaline hypersaline soda brines (Geirnaert & Laeven, 1992; DasSarma & Arora, 2001).

Information on the *Artemia* population inhabiting Wadi El-Natrun (WN) is limited. El-Sherif (1989) describes a variety of factors concerning WN *Artemia*: (1) environmental parameters, (2) biometry of cysts and nauplii, (3) adult morphometry, (4) reproductive and life span characteristics, (5) hatching characteristics, (6) essential fatty acid methyl esters of instar-I nauplii, and (7) cross-breeding experiments with *A. franciscana* (from San Francisco Bay, USA) and *A. salina* (from Megrine, Tunisia). Triantaphyllidis et al. (1997c), used allozyme and amplified fragment length polymorphism (AFLP) analyses and a morphological characteristic (i.e., the absence of spine-like outgrowths on the basal part of the penes) studied by scanning electron microscopy, to suggest that WN *Artemia* belongs to *A. salina*, and it is grouped with the bisexual populations of eastern Mediterranean basin.

In this paper, a comparative study between coastal and inland asexual *Artemia* and between asexual and bisexual strains, all from Egypt, was done based on different morphological characters, survival and growth under various salinities. These biological characteristics were recorded under standard laboratory conditions as described in Triantaphyllidis et al. (1995). The scope of this study is to present data for further characterization of these *Artemia* populations. The results may provide substantial information on the biodiversity of *Artemia* in the area of Egypt and on the potential use of these strains in saltworks operations and/or aquaculture.

Material and methods

Two parthenogenetic *Artemia* coastal populations from Borg El-Arab and El-Max, one inland parthenogenetic population from Qarun Lake and one bisexual population from Wadi El-Natrun (for *Artemia* Reference Center cyst bank code numbers and abbreviations used see Table 1) were used. All sample sites were in Egypt (Fig. 1).

Culture experiments

Cysts were incubated in artificial 0.45 μm-filtered 35 g l^{-1} medium (Instant Ocean® artificial medium – Aquarium Systems) for hatching under optimal conditions (Sorgeloos et al., 1986). After hatching, instar-I nauplii were transferred directly to five different 1 l cylindroconical flasks and the initial density was 2 nauplii/ml of culture medium. The salinity in each flask was gradually increased (by replacing part of the culture water with medium of higher salinity) to the desired level in order to avoid stressing the animals. When the salinity in each flask reached the targeted level, it was kept constant (i.e., 35, 80, 120, 150 and 200 g l^{-1}) for the rest of the experiment (Triantaphyllidis et al., 1995). Three replicates at each salinity were used. The animals were fed on a mixed diet of the yeast-based formulated feed LANSY PZ (INVE Aquaculture SA, Belgium) and *Dunaliella tertiolecta* Butcher following the feeding schedule given by Triantaphyllidis et al. (1995); this feeding regime was designed to provide high growth rates.

The animal density was reduced after day 8 to one individual per 4 ml. The temperature was 25 ± 2 °C and mild aeration was applied from the bottom of the flasks which were covered with perforated Petri dishes to minimize evaporation (oxygenation was monitored and maintained near saturation). The three replicates for each of the salinity treatments were placed in the same water bath and all experiments ran simultaneously. The photoperiod was 12 h light/12 h dark and fluorescent light tubes were used.

Table 1. List of the populations studied, their *Artemia* Reference Center (ARC) cyst bank code number and abbreviations used

Site	ARC code	Strain	Abbreviation
Borg El-Arab	1131	Pathenogenetic	BOR
El-Max	1132	Pathenogenetic	MAX
Qarun	1137	Pathenogenetic	QAR
Wadi El-Natrun	1290	Bisexual	WN

Survival rate

The total number of individuals for each population at a given salinity (in all replicates) was recorded at each water renewal (every 3 days). The results (expressed as percentages) were log-transformed and plotted against time (expressed in days). Regression analysis (Sokal & Rohlf, 1981) was performed for each population and salinity, separately. ANCOVA was used to determine if the slopes and the Y-intercepts of the regression lines were homogeneous (Sokal & Rohlf, 1981).

Growth rate

The total length in each of ten randomly sampled individuals for each population at a given salinity (in all replicates) was recorded at each water renewal (every 3 days) according to Triantaphyllidis et al. (1995): the animals were anaesthetized in chloroform-saturated seawater and measured under a dissection microscope equipped with a camera lucida and analyzed with a digitizer. The data on total length at a specific time for each population and salinity allowed the calculation of K and L_∞ parameters for the Von Bertalannfy equation (Von Bertalannfy, 1938). The equation used is in the form:

$$L_t = L_\infty[1 - e^{-K(t-t_o)}],$$

where L_t is the length of the animal at time t, L_∞ is the asymptotic length, K is the growth constant (rate of change of length increment) and t_o is the theoretical age at which $L = 0$ (Choy, 1987). K values were analyzed by applying one way ANOVA (for each salinity separately and by using the origin of the populations as a grouping factor). Tests of least significant differences (LSD) (*post hoc*) were applied in cases where differences among populations existed at a given salinity.

Morphometry

A random sample of about 30 females per flask was examined morphologically; the measurements were performed when the animals reached the adult stage. Adulthood was determined, as the occurrence of well developed egg sacs in females was obvious (whether parthenogenetic or bisexual). The following morphological parameters were examined: total length, abdominal length, maximal width of brood pouch, width of third abdominal segment, width of head, length of first antenna, maximal diameter of complex eye, distance between complex eyes, length of furca, number of setae on left furcal branch, number of setae on right furcal branch and the ratio abdominal length/total length (expressed as percentage). The animals were anaesthetized in chloroform-saturated water according to Gilchrist (1960); three drops of chloroform in 10 ml of culture medium caused the animals to be motionless for a few minutes while the heart continued beating. Recovery was complete after 10 min.

Measurements were performed under a dissection microscope equipped with a camera lucida and recorded with a digitizer (Triantaphyllidis et al., 1995).

The results were analyzed by a standard single factor ANOVA, where variances are assumed to be homogeneous (Sokal & Rohlf, 1981); Bartlett's, Hartley's, Cochran's and Levene's tests were applied to determine the homogeneity of the variance. In cases where homogeneity of variances was violated, log or square root transformations of the raw data were applied. For *post hoc* comparisons, LSD test was employed. In the rare cases that transformations did not eliminate the homogeneity of variance problem, non-parametric tests were performed (Kruskal–Wallis and Mann–Whitney).

The results were processed with discriminant function analysis (Sokal & Rohlf, 1981); this multivariate procedure computes a series of new variables (Z_1, Z_2, ...) which are linear functions of the morphological parameters considered with the form $Z_n = l_1X_1 + l_2X_2 + \cdots$ (where l_n are the calculated discriminant coefficients and X_s the variables being considered). This discriminant function is constructed in such a way that as many as possible members of one population have high values for Z and as many as possible members of the other have low values, so that Z serves as a much better discriminant of the populations than variable X_1 or X_2 does taken singly. The rationale was to identify relationships between the qualitative criterion variable (i.e., strain) and quantitative predictor variables (i.e., morphometric variables) and to identify boundaries among the four populations studied (Kachigan, 1986).

Results

Survival

The bisexual population (WN) had significantly higher survival in the salinities of 35, 80 and 120 g l^{-1} compared to this at 150 and 200 g l^{-1} (Figs 2 and 3, ANCOVA, $p < 0.05$). The maximum survival of WN strain was at 80 g l^{-1} (Figs 2 and 3); this population did not survive at 150 and 200 g l^{-1} after day 17. In contrast, the parthenogenetic strains performed significantly better at 150 and 200 g l^{-1} (Figs 2 and 3, ANCOVA, $p < 0.05$). All the parthenogenetic strains performed similarly at all salinities investigated (no significant differences were observed according to ANCOVA, see Figs 2 and 3). Comparing survival at the five different salinities, it is obvious that salinities 150 and 200 g l^{-1} separate WN population from the parthenogenetic strains.

Growth

The bisexual strain (WN) had the lowest growth rate (K) in all salinities (ANOVA: 35 g l^{-1}, $F = 303.27$, $p < 0.05$; 80 g l^{-1}, $F = 95.75$,

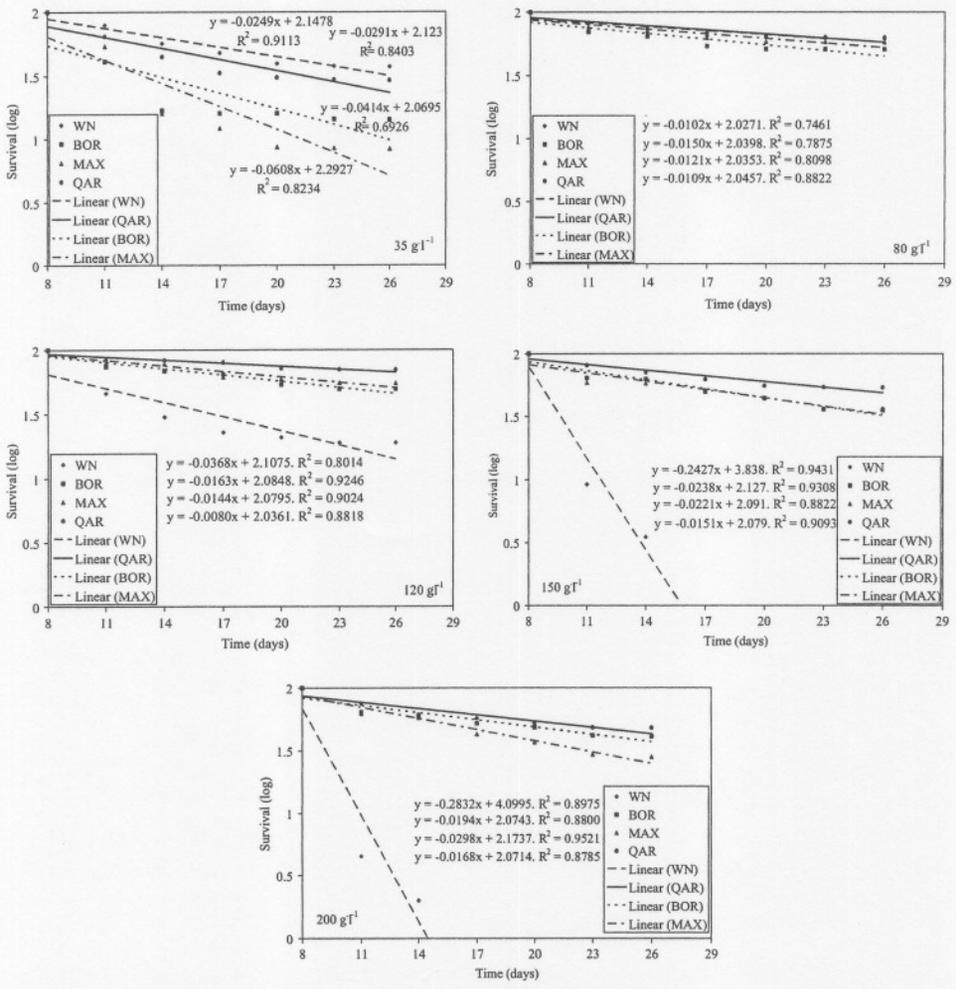

Figure 2. Survival of the four different *Artemia* strains reared at five salinities (35, 80, 120, 150 and 200 g l^{-1}). WN: Wadi El-Natrun, BOR: Borg El-Arab, MAX: El-Max, QAR: Qarun Lake.

$p < 0.05$; 120 g l^{-1}, $F = 39.32$, $p < 0.05$; and LSD test; Figs 4 and 5). The recorded maximum total length of WN was significantly smaller than this of the parthenogenetic strains (WN: 5.79 ± 0.45 mm while for the parthenogenetic strains was more than 10 mm). Growth rates of WN strain were similar at 35, 80 and 120 g l^{-1} (Fig. 5) while at 150 and 200 g l^{-1} no results were obtained, due to the high mortality after day 11 (Fig. 2). The growth of the parthenogenetic coastal strains (BOR, MAX) were, in general, similar in all salinities (according to K – Figs 4 and 5). The only statistically significant difference between MAX and BOR populations was recorded at 120 g l^{-1} (LSD test, $p < 0.05$). Maximum total length for these two strains was reached when they were cultured at 35 g l^{-1} (L_∞ was 12.98 and 12.58 mm for BOR and MAX, respectively – Fig. 4). Generally, QAR strain had lower K values as the salinity increased (Fig. 5). This inland population performed better in 150 and 200 g l^{-1} compared to coastal parthenogens (ANOVA, $F = 5.47$ and 11.76, $p < 0.05$, respectively and LSD test). BOR and MAX populations had

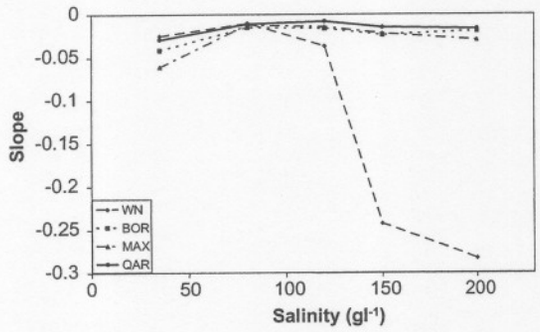

Figure 3. Regression line slope values for each population at the different salinities. WN: Wadi El-Natrun, BOR: Borg El-Arab, MAX: El-Max, QAR: Qarun Lake.

maximum K value at 80 g l^{-1} while further elevation of salinity caused a delay in their growth rates (Fig. 5).

Morphometry

The bisexual strain (WN) had significantly lower values in 7 out of 13 morphometric characters when compared to parthenogenetic strains (QAR, BOR, MAX – Table 2) in all salinities, i.e., total length, ovisac width, distance between complex eyes, length of first antenna, furca length, number of setae in left and right branch of the furca. The parthenogenetic strains BOR and MAX (both coastal populations) do not differ statistically in the majority of the morphometric variables assayed but they both differ significantly from the inland parthenogenetic population (QAR).

Discriminant analysis based on the geographical origin of each population as separator factor was performed for each salinity (Table 3 and Figs 6–10). Therefore, three roots were produced for each salinity (with standardized coefficients) except for the salinities of 150 and 200 g l^{-1}, where two roots were produced, because of the absence of WN strain. At 35 g l^{-1}, there was an absolute discrimination among the bisexual (WN) and the group of parthenogenetic strains (BOR, MAX, QAR, Fig. 6). Furthermore, the two coastal parthenogenetic strains (BOR, MAX) were grouped together and they can be distinguished from the inland one (QAR). The total predictability of the model was 75.5% (mainly because of the two coastal populations which could not be discriminated effectively due to similar values). The graph presented in Figure 6 was based on the two out of three roots that were produced, and these two roots explained 99.8% of the total variation in the data collection (see also Table 3).

The same observations were valid for 80 g l^{-1} (Table 3, Fig. 7) and 120 g l^{-1} (Table 3, Fig. 7), although the predictability of the discrimination models was higher compared to that of 35 g l^{-1} (i.e., 84.6 and 81.8%, respectively). Two out of the three roots were used to construct the graphs appearing in Figures 7 and 8 and explained more than 99% of the total variation in both cases (see also Table 3). Furthermore, it seems that the separation of the two coastal parthenogenetic strains from the inland one was more obvious at 80 and 120 g l^{-1} compared to that at 35 g l^{-1}.

At 150 g l^{-1} (Table 3, Fig. 9) and 200 g l^{-1} (Table 3, Fig. 10), the discrimination analyses were based on the two coastal (BOR, MAX) and one inland (QAR) parthenogenetic strain. The total predictability of the model was 71.1% for 150 g l^{-1} and 82.2% for 200 g l^{-1} (Figs 9 and 10), while the two roots used for constructing the graphs explained 100% of the total variation in both cases (Table 3).

The results from discrimination analysis revealed that in all salinities there was an overlapping between the two coastal parthenogenetic strains (BOR and MAX), which caused a reduction in the predictabilities. This was not the case when these two strains were pooled; the discrimination among the bisexual, inland parthenogenetic and coastal parthenogenetic strains was apparent (data not shown).

Discussion

The four populations had significant differences in their survival, growth and morphometric characteristics under various salinity regimes. Survival was affected by salinity. WN (bisexual strain) experienced high mortality as salinity increased. Best survival for this bisexual strain was recorded at the salinity range between 35 and 120 g l^{-1} while a significant drop was observed at 120 g l^{-1} upwards (Figs 2 and 3). Although, according to DasSarma & Arora (2001), some of the lakes belonging to the WN complex have soda brine

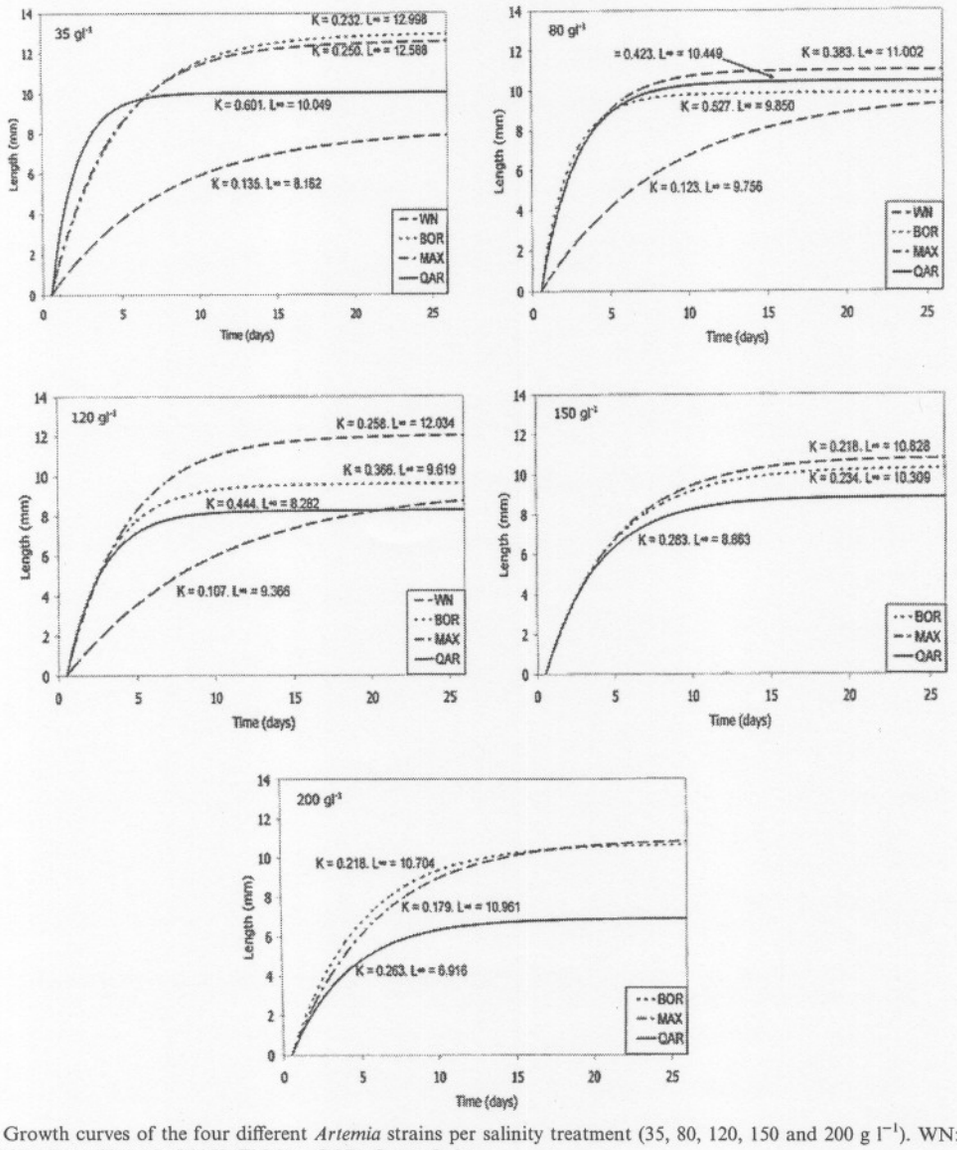

Figure 4. Growth curves of the four different *Artemia* strains per salinity treatment (35, 80, 120, 150 and 200 g l^{-1}). WN: Wadi El-Natrun, BOR: Borg El-Arab, MAX: El-Max, QAR: Qarun Lake.

waters, the athalassic origin of them (carbonate ions are prevailing) is debatable (Geirnaert & Laeven, 1992). El-Sherif (1989), based on field recordings of abiotic factors in WN lakes, reported that pH values (ranging from 7.85 to 9.12) do not support the idea of extreme alkalinity, which is typical for soda brine waters. If it was verified that WN population inhabits carbonate waters then high mortality could be attributed to the distortion of ionic composition since chloride ions are substantially increased as the salinity rose in the artificial culture medium. In order to check this hypothesis, we conducted culture experiments using carbonate-rich culture media (Van Stappen et al., 2003) but no significant differences were recorded (data not shown). The WN strain was

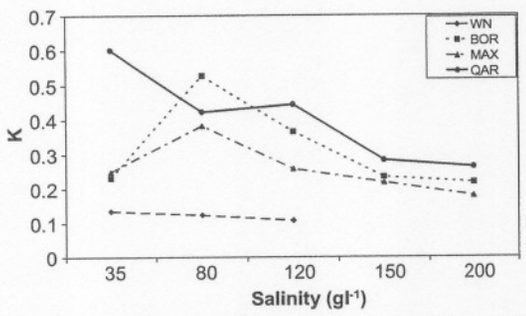

Figure 5. Von Bertalanffy *K* values for each population at the different salinities. WN: Wadi El-Natrun, BOR: Borg El-Arab, MAX: El-Max, QAR: Qarun Lake.

grouped with *A. salina* populations from eastern Mediterranean basin (e.g. Larnaca, Cyprus), as was suggested by allozyme and AFLP analyses (Triantaphyllidis et al., 1997c). Survivorship of *A. salina* from Larnaca was reported to be 0% when cultured at 60 g l^{-1} and 24 °C and increased with the elevation of salinity (Browne & Wanigasekera, 2000). This was not the case in our study and the differences may be because the WN population had adapted to water of different ionic composition (see above). Contrary to Browne & Wanigasekera (2000), Vanhaecke et al. (1984) recorded maximum survival at 22 °C and 42 g l^{-1} for Larnaca *A. salina*, and they found that its survival was low at elevated salinities (above 120 g l^{-1}). Also, El-Sherif (1989) reported that reproduction of WN strain (field observations) appeared to be highest at the low salinity levels (i.e., less than 130 g l^{-1}). El-Sherif's data are in full agreement with the interaction between reproductive performance of these strains and salinity detected by Baxevanis et al. (2004).

Artemia from WN responded differently to the elevation of salinity compared to the parthenogenetic strains. The latter performed well both at low and high salinity and, therefore, were more euryhaline than the former (Figs 2 and 3). Both inland and coastal parthenogenetic populations had similar survival in all examined salinities (Figs 2 and 3). Our results are in agreement with those of Browne & Wanigasekera (2000), who found that the survival of a parthenogenetic *Artemia* from Margherita di Savoia (Italy) increased at the salinity range between 60 and 180 g l^{-1}. In our study a correlation between salinity and survival was not evident; the parthenogenetic Egyptian populations had greater plasticity compared to the one from Italy (from 35 to 200 g l^{-1}). Triantaphyllidis et al. (1995) examined a parthenogenetic strain from Tanggu (China) and found that its survival was not affected by the elevation of salinity (up to the limit of 180 g l^{-1}).

Generally, it is believed that an obligatory parthenogenetic species should have a broader phenotypic response compared to its related sexual species based on the general purpose genotype hypothesis (Lynch, 1984; Browne & Wanigasekera, 2000). Based on the Egyptian *Artemia* populations, this hypothesis is supported with only one ambiguity: high mortality and retarded growth (see below) of WN was not due to the different water anionic composition this population experiences in its natural environment and had not a major impact on the examined characters.

For WN strain the growth performance was stable at the salinity range between 35 and 120 g l^{-1} (see *K* values in Figs 4 and 5). Its recorded maximal length was in the range of 5–5.8 mm, which means that it is one of the smallest bisexual *Artemia* ever recorded (Triantaphyllidis et al., 1997a). Our results are in agreement with those presented by El-Sherif (1989). This researcher compared WN bisexual strain with *A. salina* from Megrine (Tunisia) and *A. franciscana* from San Francisco Bay (USA) and found that WN has: (i) the smallest cyst diameter (220.1 μm), (ii) the smallest length of instar-I nauplii (399.0 μm) and (iii) the smallest adults (4.94 mm for males and 5.92 mm for females; sexually mature individuals). The comparison of growth rates for BOR and MAX reinforced by the analyses of other morphometric parameters (see below) revealed that these two coastal populations had similarities and may support the view that they belong to the same strain (Figs 4 and 5). Their recorded total length appeared to be small for parthenogenetic *Artemia* but close to other African asexual populations (e.g. Namibia – Triantaphyllidis et al., 1997b and unpublished data). Qarun population, although it originates from an inland lake far from the coastal saltworks, exhibited similarities with BOR and MAX in terms of growth rate and total length (Figs 4 and 5). All

Table 2. Mean (±SD) of various morphometric parameters of female *Artemia* for the WN, QAR, BOR and MAX populations reared at five salinities. (A) total length, (B) abdominal length, (C) width of ovisac, (D) width of the third abdominal segment, (E) width of head, (F) maximal diameter of complex eye, (G) distance between complex eyes, (H) length of first antenna, (I) furca length, (J) number of setae, left branch of the furca, (K) number of setae, right branch of the furca, (L) percent of abdomen length/total length. Populations reared at the same salinity when sharing the same letter (a–d) are not significantly different for the respective morphometric variable, as determined by parametric (ANOVA and LSD) and non-parametric (Kruskal–Wallis and Mann–Whitney) tests ($p < 0.05$). Parameters A–I are in mm.

	Salinity 35 g l^{-1}				Salinity 80 g l^{-1}				Salinity 120 g l^{-1}				Salinity 150 g l^{-1}			Salinity 200 g l^{-1}		
	WN	QAR	BOR	MAX	WN	QAR	BOR	MAX	WN	QAR	BOR	MAX	QAR	BOR	MAX	QAR	BOR	MAX
A	5.01^{a}	9.34^{b}	9.74^{b}	9.76^{b}	5.41^{a}	8.52^{b}	9.15^{b}	9.09^{b}	5.06^{a}	8.26^{b}	8.82^{c}	8.73^{c}	8.15^{a}	8.69^{b}	8.87^{b}	7.72^{a}	7.24^{b}	7.19^{b}
	(0.37)	(0.72)	(1.10)	(1.07)	(0.63)	(0.33)	(1.02)	(0.93)	(0.13)	(0.90)	(0.68)	(0.77)	(0.73)	(0.88)	(0.88)	(0.64)	(0.80)	(0.81)
B	2.05^{a}	3.57^{c}	4.17^{b}	3.79^{c}	2.25^{a}	3.43^{b}	4.31^{c}	4.20^{c}	1.97^{a}	3.60^{b}	4.31^{c}	4.21^{c}	3.48^{a}	4.07^{b}	4.04^{b}	3.24^{a}	3.31^{a}	3.32^{a}
	(0.26)	(0.49)	(0.61)	(1.01)	(0.30)	(0.36)	(0.61)	(0.59)	(0.05)	(0.35)	(0.48)	(0.53)	(0.25)	(0.51)	(0.52)	(0.33)	(0.37)	(0.46)
C	0.48^{a}	1.39^{b}	1.72^{c}	1.68^{c}	0.86^{a}	1.17^{b}	1.46^{c}	1.44^{c}	0.58^{a}	0.81^{b}	1.34^{c}	1.33^{c}	0.91^{a}	1.25^{b}	1.26^{b}	1.01^{a}	1.05^{a}	0.94^{a}
	(0.06)	(0.26)	(0.29)	(0.31)	(0.10)	(0.13)	(0.37)	(0.32)	(0.02)	(0.22)	(1.43)	(0.16)	(0.22)	(0.14)	(0.15)	(0.29)	(0.15)	(0.26)
D	0.42^{a}	0.52^{b}	0.48^{b}	0.48^{b}	0.41^{a}	0.42^{a}	0.43^{a}	0.44^{a}	0.31^{a}	0.39^{c}	0.36^{b}	0.37^{bc}	0.41^{a}	0.36^{a}	0.37^{a}	0.34^{a}	0.30^{ab}	0.29^{b}
	(0.06)	(0.06)	(0.05)	(0.07)	(0.05)	(0.07)	(0.08)	(0.07)	(0.01)	(0.04)	(0.04)	(0.05)	(0.19)	(0.04)	(0.04)	(0.12)	(0.05)	(0.06)
E	0.60^{a}	0.61^{a}	0.74^{b}	0.74^{b}	0.58^{a}	0.57^{a}	0.56^{a}	0.55^{a}	0.44^{a}	0.57^{b}	0.56^{b}	0.55^{b}	0.51^{a}	0.52^{a}	0.53^{a}	0.50^{a}	0.42^{b}	0.42^{b}
	(0.09)	(0.12)	(0.12)	(0.12)	(0.08)	(0.06)	(0.06)	(0.06)	(0.02)	(0.08)	(0.06)	(0.06)	(0.11)	(0.03)	(0.04)	(0.15)	(0.05)	(0.06)
F	0.19^{a}	0.21^{c}	0.23^{b}	0.22^{c}	0.22^{a}	0.19^{b}	0.21^{b}	0.19^{b}	0.15^{a}	0.18^{b}	0.18^{b}	0.22^{b}	0.19^{a}	0.19^{a}	0.19^{a}	0.19^{a}	0.18^{a}	0.19^{a}
	(0.02)	(0.02)	(0.03)	(0.04)	(0.03)	(0.02)	(0.03)	(0.03)	(0.01)	(0.01)	(0.03)	(0.13)	(0.02)	(0.02)	(0.03)	(0.06)	(0.01)	(0.13)
G	0.91^{a}	1.24^{b}	1.38^{c}	1.36^{c}	1.02^{a}	1.10^{b}	1.17^{c}	1.13^{c}	0.93^{a}	0.11^{b}	1.11^{b}	1.12^{b}	1.12^{a}	1.01^{a}	1.03^{a}	1.01^{a}	0.96^{a}	0.91^{a}
	(0.12)	(0.12)	(0.22)	(0.21)	(0.06)	(0.15)	(0.15)	(0.15)	(0.03)	(0.12)	(0.21)	(0.19)	(0.31)	(0.14)	(0.14)	(0.06)	(0.14)	(0.19)
H	0.42^{a}	1.06^{b}	1.10^{b}	1.14^{b}	0.78^{a}	0.97^{b}	1.05^{c}	1.05^{c}	0.49^{a}	0.90^{b}	0.96^{bc}	1.01^{c}	0.96^{a}	0.96^{a}	0.95^{a}	0.81^{a}	0.83^{a}	0.84^{a}
	(0.05)	(0.13)	(0.13)	(0.13)	(0.12)	(0.12)	(0.13)	(0.12)	(0.01)	(0.08)	(0.14)	(0.13)	(0.06)	(0.11)	(0.11)	(0.09)	(0.13)	(0.14)
I	0.13^{a}	0.47^{b}	0.40^{c}	0.36^{c}	0.18^{a}	0.32^{b}	0.30^{c}	0.35^{c}	0.15^{a}	0.29^{b}	0.29^{b}	0.34^{c}	0.28^{a}	0.22^{b}	0.21^{b}	0.15^{a}	0.22^{b}	0.18^{b}
	(0.04)	(0.15)	(0.06)	(0.06)	(0.03)	(0.04)	(0.07)	(0.06)	(0.02)	(0.06)	(0.07)	(0.06)	(0.04)	(0.04)	(0.03)	(0.04)	(0.12)	(0.02)
J	4.66^{a}	14.70^{b}	13.40^{c}	13.63^{c}	2.46^{a}	12.90^{b}	7.70^{c}	8.46^{d}	2.46^{a}	12.70^{b}	11.16^{c}	11.00^{c}	11.53^{a}	7.60^{b}	8.00^{b}	11.30^{a}	5.26^{b}	5.76^{b}
	(0.47)	(0.65)	(1.13)	(0.25)	(0.50)	(1.02)	(1.14)	(0.86)	(0.09)	(1.21)	(0.94)	(0.78)	(0.51)	(1.88)	(1.43)	(0.70)	(0.52)	(0.77)
K	4.76^{a}	15.00^{b}	13.96^{c}	14.10^{c}	2.53^{a}	13.15^{b}	8.10^{c}	8.83^{c}	2.40^{a}	13.05^{b}	11.33^{c}	11.12^{c}	11.63^{a}	7.66^{b}	8.10^{b}	11.66^{a}	5.63^{b}	6.00^{b}
	(0.43)	(0.72)	(1.21)	(0.21)	(0.77)	(1.18)	(1.37)	(0.69)	(0.09)	(1.27)	(1.09)	(1.01)	(0.49)	(1.91)	(1.47)	(0.77)	(0.71)	(0.69)
L	41.16^{a}	38.56^{c}	43.54^{b}	39.13^{c}	42.29^{a}	40.27^{a}	47.76^{b}	46.56^{b}	39.92^{a}	44.19^{a}	49.35^{b}	48.67^{b}	43.31^{a}	46.39^{a}	45.95^{a}	42.32^{a}	46.29^{b}	46.69^{b}
	(6.12)	(6.26)	(9.34)	(2.12)	(7.69)	(4.38)	(9.01)	(7.42)	(1.79)	(6.62)	(7.95)	(7.98)	(7.87)	(7.03)	(7.07)	(5.78)	(6.94)	(7.87)

Table 3. Discriminant analysis of the morphometric parameters of female *Artemia* individuals for WN, QAR, BOR and MAX populations reared at five different salinities; standardized coefficients for canonical variables, eigenvalues and cumulative percentage of variance for each root are presented

Variables	Salinity 35 g l^{-1}		Salinity 80 g l^{-1}		Salinity 120 g l^{-1}		Salinity 150 g l^{-1}		Salinity 200 g l^{-1}	
	Root 1	Root 2	Root 1	Root 2	Root 1	Root 2	Root 1	Root 2	Root 1	Root 2
A	−0.545	0.062	0.594	−1.414	−0.554	0.420	0.366	−1.148	1.133	0.610
B	0.016	0.255	0.028	0.741	−0.304	−0.936	0.066	0.936	−1.347	−0.815
C	−0.248	0.627	0.213	−0.355	−0.145	−0.886	0.403	−0.095	−0.021	−0.636
D	−0.073	−0.324	−0.118	0.033	0.044	0.137	−0.043	−0.110	−0.101	−0.511
E	−0.027	0.404	0.057	0.135	−0.093	−0.088	0.081	−0.150	0.203	−0.180
F	−0.075	0.209	−0.029	−0.008	0.085	−0.145	−0.163	−0.080	0.068	0.234
G	−0.235	0.290	0.031	−0.146	0.103	0.103	−0.293	−0.157	−0.185	−0.199
H	−0.307	0.183	0.181	−0.189	−0.242	−0.320	−0.201	−0.039	−0.126	0.212
I	−0.222	−0.345	0.378	−0.376	−0.186	−0.021	−0.687	0.615	0.240	−0.624
J	−0.387	−0.344	0.483	0.515	−0.627	0.480	−0.977	1.312	0.877	0.576
K	−0.473	−0.152	0.334	0.207	−0.151	0.155	0.169	−1.842	0.223	−0.441
L	−0.352	−0.182	0.369	−1.495	−0.298	0.961	0.369	−1.308	1.476	1.111
Eigenvalues	48.356	1.122	24.197	4.448	41.055	3.461	5.286	0.047	22.130	0.198
Cum. perc.	0.975	0.998	0.841	0.996	0.919	0.996	0.991	1.000	0.991	1.000

For the abbreviations of the variables, see Table 2.

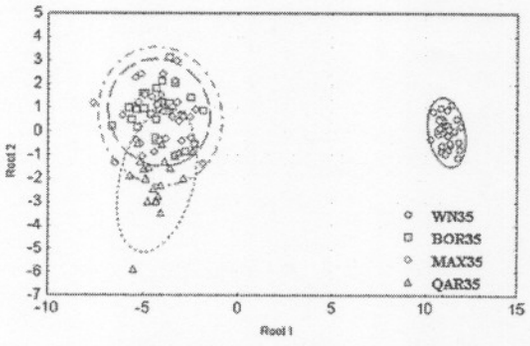

Figure 6. Scatterplot resulting from the discriminant analysis (canonical scores) at 35 g l^{-1} when using strain origin (WN: Wadi El-Natrun, BOR: Borg El-Arab, MAX: El-Max, QAR: Qarun Lake) as separator factor. Total predicted classification was 75.5% (WN: 100.0%, BOR: 63.3%, MAX: 56.7% and QAR: 85.0%). Border lines represent 95% confidence level.

parthenogenetic strains scored in this study grow slowly as the salinity increases (see Fig. 5), which is congruent with the results from other *Artemia* populations examined (Triantaphyllidis et al., 1995).

The Von Bertalannfy growth model has been applied successfully in two different species of Anostraca (*Branchipus schaefferi* and *Streptocephalus torvicornis* – Beladjal et al., 2003). *K* values for the females of these two species are smaller than those reported in this study for *Artemia* populations (0.106 and 0.033 for *B. schaefferei* and *S. torvicornis*, respectively). Discriminant analysis proposed by Hontoria & Amat (1992a, b) and used by other authors (Amat et al., 1995; Triantaphyllidis et al., 1995, 1997a, b) can provide significant information for the distinction of two or more groups. Furthermore, a successful discriminant analysis can be a useful tool for assigning an unknown individual to one of the known groups, which could be useful for management purposes or biodiversity studies. If the morphometric analysis reveals the existence of two or more different groups within the population then more accurate techniques (such as allozyme, RAPD, RFLP, AFLP analyses, or combinations of those) should be applied for the complete characterization of the groups (Triantaphyllidis et al., 1997c; Gajardo et al., 1999; Sun et al., 1999a, b; Abatzopoulos et al., 2002a, b). Therefore, the need of morphological and genetic databases containing as much information as possible is crucial.

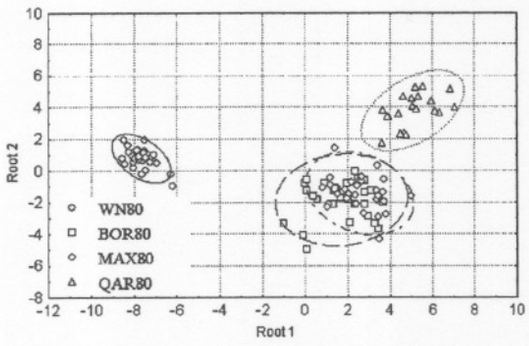

Figure 7. Scatterplot resulting from the discriminant analysis (canonical scores) at 80 g l^{-1} when using strain origin (WN: Wadi El-Natrun, BOR: Borg El-Arab, MAX: El-Max, QAR: Qarun Lake) as separator factor. Total predicted classification was 84.6% (WN: 100.0%, BOR: 66.7%, MAX: 76.7% and QAR: 100.0%). Border lines represent 95% confidence level.

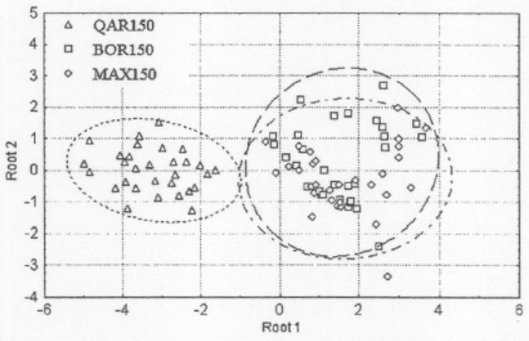

Figure 9. Scatterplot resulting from the discriminant analysis (canonical scores) at 150 g l^{-1} when using strain origin (BOR: Borg El-Arab, MAX: El-Max, QAR: Qarun Lake) as separator factor. Total predicted classification was 71.1% (BOR: 56.7%, MAX: 56.7% and QAR: 100.0%). Border lines represent 95% confidence level.

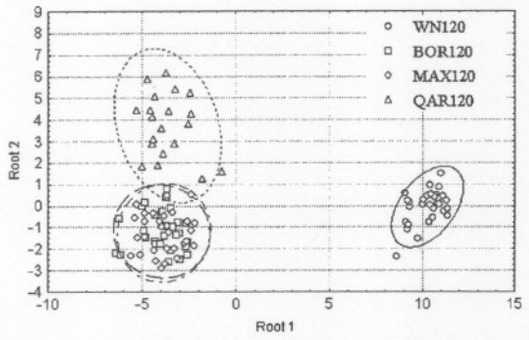

Figure 8. Scatterplot resulting from the discriminant analysis (canonical scores) at 120 g l^{-1} when using strain origin (WN: Wadi El-Natrun, BOR: Borg El-Arab, MAX: El-Max, QAR: Qarun Lake) as separator factor. Total predicted classification was 81.8% (WN: 100.0%, BOR: 70.0%, MAX: 66.7% and QAR: 95.0%). Border lines represent 95% confidence level.

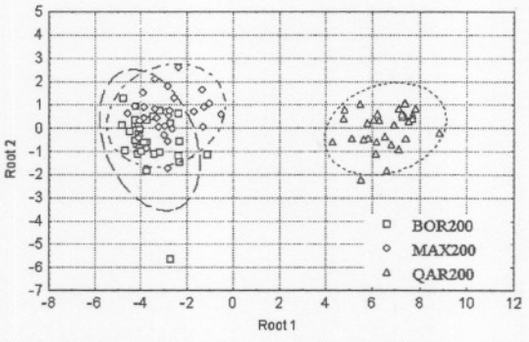

Figure 10. Scatterplot resulting from the discriminant analysis (canonical scores) at 200 g l^{-1} when using strain origin (BOR: Borg El-Arab, MAX: El-Max, QAR: Qarun Lake) as separator factor. Total predicted classification was 82.2% (BOR: 73.3%, MAX: 73.3% and QAR: 100.0%). Border lines represent 95% confidence level.

At salinities of 35, 80 and 120 g l^{-1} (above 120 g l^{-1} only parthenogenetic populations could survive, see Fig. 2) discrimination models resulted in overall predictability of 75.5, 84.6 and 81.8%, respectively (Table 3; Figs 6–8). It should be noted that the predicted classifications for WN strain reached 100% at each salinity and therefore, it can be separated from the parthenogenetic populations at the salinities of 35, 80 and 120 g l^{-1}. However, discrimination among parthenogenetic strains (BOR, MAX and QAR) at these salinities was not sound; only at 80 g l^{-1}, QAR predicted classifications were 100%. At 35 and 120 g l^{-1} QAR was slightly mixed up with BOR and MAX (Figs 6 and 8). These last two strains produced very low predicted classification percentages and the discriminant analysis failed to separate them (Figs 6–8). This was due to similar values, in the majority of the morphometric variables examined, as expected, since they are both from interconnected coastal saltworks. The results from discriminant analysis revealed that the morphometric variables, which differentiate the bisexual from the parthenogenetic populations, were mainly the total

length, number of setae in the left branch of the furca and the abdominal length. These variables were significantly lower in WN compared to QAR, BOR and MAX (Table 2).

According to our results, the best salinity for culturing *Artemia* strains from Egypt in order to discriminate individuals and assign them in recognizable groups is 80 g l^{-1}. At this salinity, there is discrimination between the bisexual (WN) and parthenogenetic strains and among coastal (BOR, MAX) and inland parthenogenetic (QAR) populations.

The results revealed that there was an excellent discrimination at 150 and 200 g l^{-1} between the two coastal strains (BOR and MAX) and the inland one (QAR). The discrimination between the two coastal strains was not possible although elevated salinities of 150 and 200 g l^{-1} gave 56.7 and 73.3% of predicted classifications (Figs 9 and 10; Table 3). The morphological parameters that contributed mostly to the discrimination of asexual populations were the total length and the number of setae in each branch of the furca. QAR strain seemed to be smaller in size compared to BOR and MAX at 150 g l^{-1}, but the opposite was recorded at 200 g l^{-1}. Also, QAR had a higher number of setae in both branches of the furca than BOR and MAX at both salinities (Table 2).

Although salinity affects the number of setae, it can be used as a reliable discriminating character among the populations studied (Table 2). Also, Amat et al. (1995) recognized that the size of furca and the number of setae varies considerably in wild populations, due to environmental conditions. Nevertheless, the same researchers acknowledged that these morphological variables can be trustworthy as a systematic tool, provided that well defined culture conditions are followed. They found that furcal characters were the major factors for discriminating a group of Southern Spanish populations from the rest of the Spanish bisexual *Artemia*.

Even though the salinities of 150 and 200 g l^{-1} could partition WN from the parthenogenetic strains, these salinities should be avoided for culture purposes; survival at these salinities for parthenogenetic strains does not exceed 60% and therefore a substantial part of the population is not represented. However, our results verify that the parthenogenetic strains have greater phenotypic plasticity and can tolerate a broader range of salinities (Triantaphyllidis et al., 1995) than sexual strains.

Triantaphyllidis et al. (1995) examined a parthenogenetic strain from Tanggu (China) and *A. franciscana* and concluded that a discriminant function could predict with an accuracy of almost 100%. Furthermore, Triantaphyllidis et al. (1997b) examined 15 parthenogenetic *Artemia* populations, and they produced a discriminant analysis which had an overall prediction accuracy of 93.2%. Amat et al. (1995) used discriminant analysis that was based on morphometric variables to assign a population from Veldrif (South Africa) to Mediterranean sexual populations rather than to other known bisexual *Artemia* species. Our results verify the role of discriminant analysis to characterize a strain and this work could be another contribution for constructing a database of morphometric characteristics.

The following conclusions may be drawn:

- The two coastal parthenogenetic strains (BOR and MAX) may be assigned to the same population since they were similar in terms of survival, growth and morphometry at various salinity regimes.
- The parthenogenetic Egyptian populations (QAR, BOR and MAX) are more euryhaline than the bisexual one (WN), although it is essential to stress that the ionic composition in WN natural environment is most probably different from that of the culturing medium. This is mostly apparent at 150 and 200 g l^{-1}.

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